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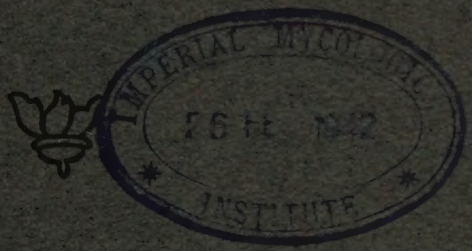

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SYSTEMIC INFECTIONS OF RUBUS WITH THE ORANGE-RUSTS[1]

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INTRODUCTION

Cultivation of some of the most desirable varieties of blackberries and raspberries has been limited or discontinued in certain regions because of the orange-rusts which inhibit the development of fruit on the canes infected. Local infection of leaves of blackberries by sowing aecidiospores of the long-cycled orange-rust, Gymnoconia, has been brought about by a number of persons. Reference to such work as has been done along this line has been made by Clinton (*3*, *4*)[2] and Kunkel (*7*).

The problem of working out practical methods for the control of these rusts, however, depends upon a knowledge of when and how the gametophytic mycelium enters the host and becomes established as a constitutional parasite. The results of infection experiments dealing with this problem are reported at this time.

The common blackberry has perennial roots from which canes arise year by year, those of one year bearing fruit the next. The turions appear in the spring from the buried crown or from root runners and become the "old canes" of the next year, dying after fruiting. The new canes are of the type having indefinite growth; therefore the formation of a terminal bud which would remain dormant during the winter and push out into new growth the following spring would be very unusual and abnormal, although lateral buds at the lower part of the old cane frequently develop into new branches. The crown usually lies a few inches beneath the surface of the soil, and consequently there is a part of the original cane which lives several years. As new shoots and roots arise from this structure it loses its identity. Nurserymen would refer to that part of the cane beneath the soil simply as the crown because new canes arise in this region. It may also be referred to as the perennial base. Figure 1 shows these features diagrammatically for a simple plant (see also fig. 5). The structure of the cane and the relation of its tissues as they appear in cross-sections are brought out in Plate 2, B.

HOST PARASITE RELATIONS

In dealing with a rust which is perennial and systemic in a woody plant, it is desirable to be familiar with the appearance and distribution of the mycelium. The sporophytic hyphae of the Gymnoconia are con-

[1] Accepted for publication May 2, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 242.

fined to the leaves in localized areas. The gametophytic hyphae become established in the perennial plant structures, from which they invade the new canes throughout their length and finally take part in the formation of aecidiospores. Newcombe (*8*) and Clinton (*3*) have given practically the only accounts which deal with the mycelium and haustoria of the orange-rusts in the host tissues. Both authors used the infected blackberry as the subject of investigation. Newcombe did not study sections of the roots, confining his attention to 16 cm. of an infected blackberry cane. He discovered that the bulk of the mycelium was to be found in the pith of the stem, noting hyphae in a medullary ray at one place. Clinton found mycelium in the fundamental tissue of the growing point, and although in old stems the hyphae were confined to the pith, they sometimes occur in the cortex and in the phloem of the bundles in young and growing shoots. He states that—

> mycelial threads are present from the upper parts of the roots running through the stem into the uppermost leaves showing signs of infection * * *. Frequently plants are found in which the new shoots are affected but the old ones are free. In such case the mycelium is found in the former only * * *. Sections of roots, except in the neighborhood of the merging of root and stem, do not show the mycelium.

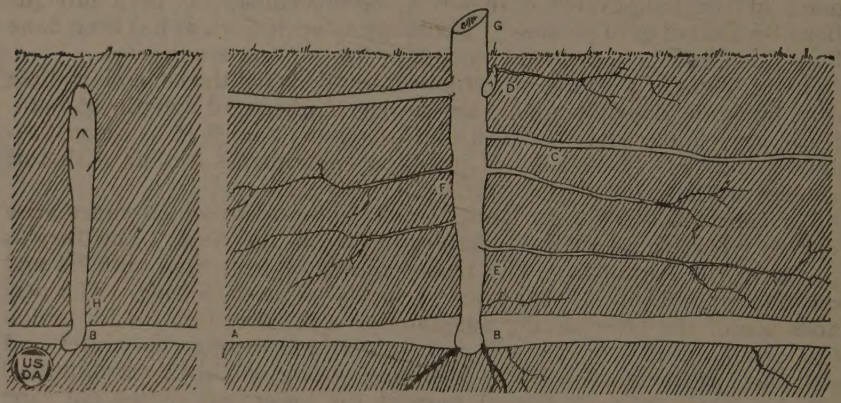

FIG. 1.—At the left, growth of shoot H from root runner, A, in April. At the right, same plant in October; B, root crown; small central pith would be found at E; horizontal roots, C, which happened to develop in this case do not ordinarily appear the first year.

Intercellular hyphae without septa or nuclei and the simpler types of haustoria are figured.

MYCELIUM IN THE BLACKBERRY

Clinton's account of the distribution of the mycelium is correct in general if applied to blackberries which have been infected for at least two years, except when he states that the roots of an infected plant do not show any mycelium other than in the transition zone where root and stem join. The writer has found that hyphae invade the roots of the blackberry very extensively, a fact which accounts for the rapid spread of the parasite to new shoots which rise from the roots. The blackberry forms a few large roots, some of them becoming runners which by budding give rise to new plants at some distance from the parent. These runners are true roots morphologically, their structure being the same as that of the ordinary root of the blackberry. If one carefully uproots rusted

blackberries growing together in nature, he often finds several infected plants attached to the same root. The writer has made sections of such connecting root runners and has found an abundance of mycelium in the medullary rays, in the phloem near the cambium, and in the cortex for many feet, or as far as the runner extends. Ordinarily but little mycelium will be found in the woody portion of the roots except along the medullary rays. More rarely hyphae are scattered irregularly in the wood; this usually occurs in the case of recent primary infection of root shoots by spores where there has been an increase in the amount of porous wood tissue formed as the result of the late invasion of the root by the parasite.

The distribution of the mycelium was traced in the canes and in the root system of a certain colony of infected wild blackberries at the time of year when rust pustules were maturing. There were found at a point (A in fig. 2) the remains of dead canes in the form of a witch's broom characteristic of plants infected by this rust. Large roots extended downward into the soil. From one root which was of the runner type

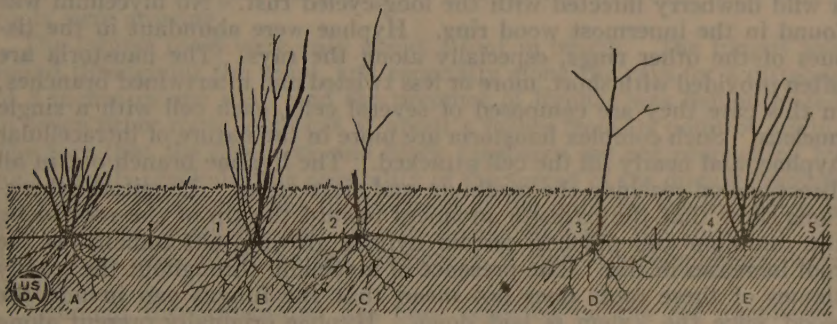

FIG. 2.—Diagram of a series of wild blackberry plants April, 1921, arising from the same root runner (part shown here about 6 feet long). A, dead canes of 1919 plant in form of a witch's broom; B, old cane (heavy line) and new shoots showing rust; C, stub of dead cane, new shoot rusted; D, old cane in blossom, not rusted; E, witch's broom consisting entirely of new shoots; sections of the regions 1, 2, 3, 4, and 5 of the root showed hyphae in the phloem and cortex. The plant D escaped infection through some accidental failure of the root mycelium to penetrate the shoot bud in time.

new shoots and old canes (B), now rusted, were arising at a distance of 18 inches. At C, 10 inches beyond, another plant, composed of rusted shoots, was attached to the same root; the old cane had died. Two feet farther (D) the plant from the runner consisted of one old cane in blossom and free from rust. At E, 14 inches distant, was a witch's broom of infected new shoots. Sections were made of the root runner at points on both sides of each plant; mycelium was found in the phloem and cortex in every case, demonstrating clearly the method by which the rust spread through the root system. Hyphae have been found in the ordinary type of root 18 inches below the ground. A plant arising from a runner bearing mycelium may rarely be devoid of rust (plant D noted above) due to the failure of the mycelium to enter the shoot at the time of its origin, just as rust-free canes are found in an infected hill. If the rust enters a shoot bud from underground parts, hyphae will later be found only in the pith of the cane, except at the nodes. In these regions hyphae may grow along the rays or outside of the wood ring.

The blackberries studied in the vicinity of Washington, D. C., and at Cameron, N. C., were infected with the short-cycled rust. Specimens of

Rubus canadensis, mountain blackberry, from Bartlett, N. H., infected with the long-cycled Gymnoconia show that the gametophytic mycelium of this rust spreads through the root runners in the same manner as that of the short-cycled form.

MYCELIUM IN THE DEWBERRY

The rust spreads in the wild dewberries in a totally different way. The trailing vines of the dewberry take root at the nodes or at the tips, and the mycelium in an infected plant, though confined to the pith in the differentiated region of the vine, follows the growing point and enters the buds as they are formed, so that new plants originating at these rooting nodes are infected from the beginning. Hyphae are present in that part of the stem which is buried in the soil, and, contrary to what might be expected, there is considerable extension of the mycelium in the root system. Sections of a dewberry infected with the short-cycled rust showed mycelium in the roots for a length of at least 8 to 10 inches.

The course of the mycelium was also followed in the root system of a wild dewberry infected with the long-cycled rust. No mycelium was found in the innermost wood ring. Hyphae were abundant in the tissues of the other rings, especially along the rays. The haustoria are often provided with short, more or less twisted and intertwined branches; in this case they are composed of several cells, each cell with a single nucleus. Such complex haustoria are more in the nature of intracellular hyphae, and nearly fill the cell attacked. The hyphae branch out in all directions on reaching the cambium and sieve tubes. Longitudinal sections show that the parasite advances toward the tip of the root along the cambium and phloem, certain hyphae growing out radially as the root increases in size. The presence of mycelium throughout the woody tissues of these roots does not mean that the fungus can invade the wood, once the xylem is laid down. Hyphae originally present along the inner side of the cambium layer are simply cut off, or surrounded, by new wood cells. The mycelium now forms an intricate network by which hyphae in the phloem and the living medullary rays are in direct connection with that part of the mycelium in the wood, so that it is not surprising to find hyphae fully alive embedded in the wood of old roots. Passing from the primary to the secondary and tertiary roots, the mycelium is more and more confined to the outer part of the wood cylinder and phloem, until in the smaller rootlets 1 year old the writer found hyphae only in the soft tissue outside of the wood cylinder—that is, along the cambium, phloem, and in the cortical parenchyma—indicating that practically all forward growth of the fungus takes place in the latter tissues. Certain roots of an infected plant may escape invasion by mycelium, and rust-free vines in such hills are not rare.

MYCELIUM IN BLACK RASPBERRY

Theoretically, the habit of the black raspberry cane which enables it to develop into a stolon which roots at the tip ought to serve admirably for the distribution of the long-cycled rust to new plants. In order to learn whether this were true, several rusted wild black raspberries have been marked and observed for three years. Not infrequently the rusted plants are so severely attacked as to die the second or third year. Again, the canes in the infected plants are of the witch's broom type and they

are not apt to become stolons and take root. In such plants the disease "runs out" because its host dies. Occasionally, some of the infected canes recover vigor and become stolons, in which case the mycelium passes from the stolon into the buds which arise to make new shoots at the point of rooting, thus spreading the disease to new plants. Sections of the internodes of infected canes and of such stolons have always shown mycelium in the pith, none in the phloem or cambium. Further consideration will be given this point in another connection. The distribution of the hyphae in the underground parts of the infected black raspberry is practically the same as that found in the blackberry and dewberry, except that hyphae are very much more abundant in the woody tissues of both roots and stems of the raspberry. One black raspberry examined had been under observation for three years. Sections of the large vertical underground stem bases from which the spindling canes were now arising, showed mycelium in the pith, scattered irregularly through the wood and along the medullary rays in the xylem and phloem regions. No hyphae were found in the well-marked pith of the much enlarged and distorted rhizomelike structure from which roots were growing.

The largest roots were only about 1 foot in length. The fungus was found throughout the wood, and especially along the rays for a distance of 8 inches in one root; and the presence of hyphae was demonstrated in seven other roots, each cut at about 3 inches from its point of origin. The fact that the dewberry and black raspberry are not propagated by root sprouts precludes the possibility of the spread of the disease through the roots. It is clear that the invasion of the roots by the mycelium is a matter of nutrition and is not correlated with the spread of the disease, as one might be led to believe if only the blackberry were studied.

Although the orange-rust has been reported on the red raspberry, *Rubus strigosus*, the writer has been unable to obtain specimens for an investigation of the distribution of the mycelium of the systemic stage in this host.

LOCAL INFECTION OF RUBUS WITH AECIDIOSPORES OF THE LONG-CYCLED RUST, GYMNOCONIA INTERSTITIALIS

In order to obtain a supply of teleutospores for experimental purposes, it was found desirable, for example, to infect the black raspberry by sowing aecidiospores of the Gymnoconia from this host. It is well known that young apple leaves are much more liable to infection by the cedar-rust than are the older or more mature leaves. It was with this in mind, as well as to obtain shorter plants for inoculation, that the writer at first purposely pruned away the old canes in the potted plants used in the greenhouse work. Plants in nature having young shoots on which leaves were just unfolding were chosen in preference to those with the old canes present or with leaves that were more fully grown. Judging from the results which followed, both practices were unwise, since it was found that the leaves on the old canes and the lower leaves on the new canes frequently bore telia, while the leaves which were just unfolding at the time the spores were sowed rarely became infected. The experiments were repeated the two following years, and in each test the tip ends of the raspberry canes were tagged at the points where the leaves were just beginning to unfold. Below the tags the leaves were fully expanded. Furthermore, the old canes were not cut away,

so several types of leaves with respect to age and position on the canes were exposed to infection. The leaves on the old canes were the most susceptible each year.

Leaves of the black raspberry were infected with the telial stage by sowing aecidiospores from the blackberry, and the southern dewberry, *Rubus enslenii*, was likewise infected by sowing aecidiospores from the black raspberry. Other species or varieties of Rubus said to be immune, or at least very resistant, to the orange-rust stage were infected with the telial stage.

The conditions under which leaves of Rubus are most readily infected with the sporophytic or "*Puccinia peckiana*" stage is outside the scope of this paper. This phase of the work is being considered in another paper on the effect of orange-rust on the development and distribution of stomata

SYSTEMIC INFECTION OF RUBUS OCCIDENTALIS WITH SPORIDIA OF THE LONG-CYCLED RUST

In infection experiments with the systemic stage of these rusts, the ordinary means of checking results by the use of isolated control plants alone does not provide sufficient safeguard. No one can explain or account for the results reported by Atkinson (*2*) on any other basis. A blackberry or raspberry may be infected in its underground parts without showing the rust on the aerial parts every year, so it would be unsatisfactory to use as experimental plants those which have just been received from a nursery or have been dug up in nature. An infected plant may be transplanted during the spring before the leaves are out and develop leaves normally in the greenhouse without showing rust that season, especially if it recovers only slowly from the shock of transplanting. Since the presence or absence of mycelium in a cane or root can be demonstrated without question, one can be certain by using only tip shoots obtained from rooting canes that the raspberry which he inoculates is uninfected at the beginning of the experiment. It is not necessary to provide control plants in checking up the results.

METHODS

The methods used in preparing sections of canes or roots to demonstrate the presence of mycelium are the same as those adopted in the writer's work on Gymnosporangium. Transverse sections 10 to 50μ thick stained with acid fuchsin and iodin green show hyphae and haustoria very plainly under the microscope. Longitudinal sections show hyphae much better, especially where they burrow along between the cambium and the phloem—for example, near the nodes of blackberries which have been primarily infected. It is very easy to overlook the hyphae in the cambium region if one does not prepare longitudinal sections.

The habit of the black raspberry mentioned above, whereby the canes take root at their tips, provides a very efficient natural method of propagation. Since it is possible to examine these stolons to learn whether or not the fungus has in some way established itself previously in the plant to be used, it has been found advisable for purposes of inoculation to use the tip plants.

The tip ends of some of the raspberry canes were wired to the soil in ordinary flower pots as soon as they began to mature sufficiently to take root. After a few days, leaves of black raspberry bearing teleutospores were laid over the rooting tip ends and kept moist under muslin damp chambers for several days.

INFECTIONS IN THE GREENHOUSE

About the middle of January the inoculated plants were transferred from the cold frames to the greenhouse. As soon as new shoots appeared it was evident that a number of the experiments had been successful. Fifteen plants which had been propagated by rooting the tips of rust-free plants were inoculated during August and September, 1921. Nine of these plants developed aecidia the following spring. An examination was made of the cane from which each new plant had been derived, material for sections having been obtained at the time the new plant was separated from its parent. No hyphae were discovered in any of these canes. As a double check when the rust began to show, another set of sections was made of that part of the original stolon still attached to the new plant. Sections were also made of the tip ends of such canes as had grown forward after they had taken root, so it is evident that the nine plants now showing rust were uninfected at the beginning of the experiment. The parasite must have gained entrance in each case as the result of inoculation with

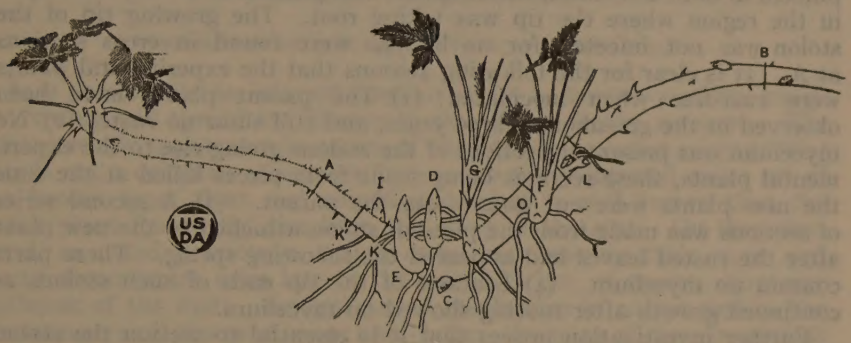

FIG. 3.—Diagram of rooting tip of black raspberry, *Rubus occidentalis*, No. 215, which was infected by laying leaves bearing telia over the tip. Shoots G and F bore pycnia at this time. Shoot buds C, D, E, and I were infected, but still beneath the soil; L, dormant bud. Sections at A and B showed no traces of mycelium. Sections of the stolon at L, O, K, and K' showed hyphae along the cambium and phloem, none in the pith. The young shoot buds, C and I, had hyphae in pith as well as in the growing region and among the bundles where differentiation was incomplete.

sporidia from teleutospores and not from hyphae carried over from an infected parent plant. It was pointed out previously (p. 213) that in the internodes of an infected cane the mycelium is confined to the central pith. At or near a node, hyphae are sometimes found growing along the medullary rays in the wood and in the cambium and phloem regions. In any case, there will always be some mycelium in the central pith. It is due to this characteristic distribution of the hyphae that it is possible to distinguish between cases of primary infections by sporidia and secondary infections where the mycelium enters a new cane directly from the infected parent plant.

On February 18 one of the artificially infected plants, No. 215, was freed from soil and photographed (Pl. I), and a diagram (fig. 3) was made in order to locate the regions or structures from which material was obtained for sections. The new shoot buds, figure 3, C, D, and E, 3 mm. to 1½ cm. long, were pushing out, but had not reached the surface of the soil and were still without chlorophyll. At L there was a dormant axial bud near the base of which a few roots were attached. Sections of the stolon at B and of the roots and dormant bud at L showed

no hyphae, but several hyphae having haustoria could be seen running between the cambium and phloem of the cane near the point of origin of this bud. It is clear that if the bud had developed it would not have been infected because of the very effective barrier of indurated tissue which was present between the hyphae and the bud. The next year an uninfected cane would have been seen in a hill where the other canes were rusted. The location of the mycelium in the stolon was also determined by sections at O, K, and K'. In each place the hyphae were confined to the region of the cambium and phloem, being absent in the pith wood and cortex. Sections of the basal portions of the infected shoots F, I, and C showed that the mycelium is much more generally distributed in the pith, phloem, and cortex of these young structures. The absence of mycelium from all tissues at A and B and in the dormant bud L, taken in connection with the general distribution of hyphae in the shoots, indicates that this plant was first infected in an axial bud which later gave rise to a shoot such as F. The mycelium after entering the tissue at the base of the bud made its way along the cambium and phloem in both directions, entering new bud primordia as they developed in the region where the tip was taking root. The growing tip of the stolon was not infected, for no hyphae were found in cross sections at A. It is clear for the following reasons that the experimental plants were rust-free when inoculated: (1) The parent plants have been observed in the greenhouse three years, and still show no rust. (2) No mycelium was present in sections of the stolons giving rise to the experimental plants, these sections being made from pieces killed at the time the new plants were cut away from the parent. (3) A second series of sections was made from the piece of stolon attached to the new plant after the rusted leaves had appeared the following spring. These parts contain no mycelium. (4) Sections of the tip ends of such stolons as continued growth after rooting showed no mycelium.

Further investigation proves that it is essential to section the stolon only at the place where it has taken root or where the infected shoots originate, in order to determine whether a rusted tip-plant has been primarily infected by sporidia, or has been invaded by hyphae from its infected parent. If there are no hyphae in the central pith of the parent cane where it roots one can rest assured that it is a case of infection by sporidia. The mycelium will then be found along the cambium and phloem.

During the time in which the tip of the stolon is establishing a root system, buds are also developing. Since the fundamental parenchyma of the bud primordium at this time is not well protected with scales and the wood ring is still unformed, hyphae from spores falling on these parts might easily find their way into the growing regions and penetrate to practically all parts of these buds, and from here grow back along the cambium region. The cambium and phloem tissues are especially well provided with food which enables the fungus to establish itself outside of the xylem ring in the phloem or cortex, from which it readily makes its way into the new buds as they are formed in this region.

The plants that escaped infection in the writer's inoculation experiments appear to be the ones in which the stolon after taking root grew on to some extent without forming shoot buds, so no means was afforded at that time for the entrance of the fungus into the host. The growing tip of a stolon is certainly a very tender structure which one would think might be attacked by the parasite. If this happened in any of the ex-

periments the tip must have stopped growing forward or died, since in every case in which the tip grew beyond the rooted plantlet, it contained no mycelium. Certain stolons rooted early so that the shoots were of considerable size before teleutospores were available and were undoubtedly too old to become systemically infected.

The experiments, described above by which tip plants were systemically infected were repeated during the summer of 1922. Teleutospores for this work were obtained from blackberry leaves. This attempt to infect the raspberry with the strain from blackberry proved successful; four of the tip plants showed orange-rust the next spring.

FIELD EXPERIMENTS

Leaves bearing teleutospores were laid over rooting tips of a number of wild raspberries in the field. The rainfall in this region was so limited in August and September, 1921, that these rooting tips rarely formed buds or shoots until the following spring. Only one plant was infected in this way.

PRACTICAL CONSIDERATIONS

PROPAGATION OF NEW PLANTS FROM DISEASED STOCK

It is possible for an infected raspberry cane to become a stolon which by rooting will spread the disease to new plants in the nursery. The characteristic appearance in nature of plants heavily rusted suggests that this is not a common occurrence because so many infected canes are of the broom type, short and spindling; and it is well known that the entire plant frequently dies from the effect of the rust. On the other hand, as noted previously, rusted canes may recover their vigor and many shoots which arise late in the season do not, of course, show any outbreak of the rust. Several rusted raspberries in nature and in the greenhouse were observed in 1921 and later. In many of these no stolons were formed, but occasionally canes clearly diseased did take root at the tips and gave rise to new plants. In several such cases the rooted plants were cut away from the parents and pieces of the stolons were sectioned. It was found in each case that the mycelium was present in the pith of the cane which had rooted at the tips, and that it must have entered the buds from which the new plants developed because they showed the rust the following spring.

RUST-FREE PLANTS FROM INFECTED STOCK

One commonly observes that some young canes in an infected hill do not show rust. When no mycelium can be found in such canes, it is clear that they arise from a part of the crown into which the fungus has not penetrated. These canes taking root at the tips must give rise to perfectly healthy plants. An infected plant was brought from Massachusetts, in June, 1920. A stolon was formed after the plant had apparently recovered from the rust. This stolon was examined after it had taken root at the tip, but no mycelium was found. The new tip plant showed no rust in 1921. It would be bad practice in a nursery to use such plants for stock, because, as noted above, mycelium may exist in a cane which shows no signs of the rust at the time. New plants arising from rooting stolons may be readily infected in August and September, but if the tip

plants have grown to considerable size before inoculation is possible, infection is unlikely to occur, This being known, methods can be easily worked out for the insuring of propagation of rust-free nursery stock.

TIP PLANTS INFECTED IN NATURE

If it is possible to infect "tip plants" of *Rubus occidentalis* experimentally with the systemic stage of the rust, what is the evidence that this is the method by which the disease is spread in nature? If one examines in early spring wild black raspberries in a region where infected plants are known to have existed in the past, he will find rusted tip plants arising from stolons which are connected with rust-free parents. Sections of such old stolons will show no hyphae. This originally suggested to the writer the probable method of primary infection.

OLD PLANTS SUBJECT TO INFECTION

There remains a question as to whether the rust is able to gain entrance into an old plant. The writer's experiments along this line have not been as satisfactorily checked as is desirable. Eight plants were obtained from a region where rust was known to be present. They showed no rust in 1921 after they were planted in the greenhouse. Since they were very small and had new shoots starting out from the old crown at the time when the first teleutospores were available, they were placed under favorable conditions for infection. Having been overwintered in the cold frame they were again brought to the greenhouse, where shoots bearing pycnia made their appearance on three of the plants within a few days. Apparently the inoculations had been fairly successful, but one can not be certain that the plants were not infected at the time they were transplanted.

In nature the new canes spring up from the base of old plants early in the season, so the gametophytic stage of the long-cycled rust naturally attacks the tip plants, which are in the most susceptible condition from the latter part of July to September, and not the basal shoots from old canes which arise in the spring. It should not be forgotten that teleutospores sometimes mature on leaves of the old canes a very short time after aecidiospores are shed, no doubt early enough to lead to infection of the more tardily formed basal shoots. It is not an uncommon practice among growers to prune out all the old canes and some of the new ones after the crop is harvested. New shoots might, as a result, grow up from the base of the old plant following this treatment, so that the conditions would be favorable for infection by sporidia from telia which would be mature at this time. There is still another possibility. The teleutospores of Gymnoconia are known to be capable of germination as soon as mature. The writer's experiments prove conclusively that some of these spores germinate in August and September if placed under suitable conditions. On the other hand no one has proved that the teleutospores may not also live over winter and be in condition for germination as the first new buds or shoots break through in early spring.

It has been pointed out that rusted canes do not ordinarily become stolons and set tip plants, so the rust is not spread in this way to any great extent. New shoots arise from the base of the old plants in the spring, while the teleutospores mature and germinate from July to September, the same time that the canes root at the tips. It must be concluded, therefore, that in nature the perennial stage of the long-cycled

orange-rust on black raspberry ordinarily spreads from plant to plant by means of sporidia which infect the rooting tips of canes in August and September.

SYSTEMIC INFECTION OF THE BLACKBERRY WITH SPORIDIA OF THE SHORT-CYCLED FORM

There are clearly two strains of orange-rust on blackberries, the short-cycled form, "Kunkelia," and the long-cycled form, Gymnoconia, which has *Puccinia peckiana* as its teleutosporic stage. The writer is describing elsewhere blackberries that are infected with orange-rust of such a nature that spores in one aecidium all develop promycelia, and those in some adjacent aecidium all produce germ tubes. One should perhaps hesitate before adopting separate generic names even for the strains that appear to be well fixed. It certainly is to be regretted that the aecidiospores of the "Kunkelia" are sometimes referred to now as "teliospores." This practice not only leads to confusion but has no basis in morphology. The pycnia of the short-cycled rust are said by Arthur (*1*) to be subcuticular. They are, of course, sub-epidermal, the same as are the pycnia of the Gymnoconia. Newcombe (*8*) and Clinton (*3*) illustrate sub-epidermal spermogonia of the orange-rust.

METHODS

During April and the first weeks in May, blackberries at the Arlington Farm, Va., send up shoots in large numbers from roots. By digging about shoots that have appeared above the ground, one can find white or reddish shoots in still earlier stages of growth. Some of these young shoots may be broken off or injured as one endeavors to uncover the youngest, but he can readily find places where a half dozen or more ranging from mere buds to shoots 6 or 8 inches high, are growing in a space covered by the infection frame. A large pan is set on sticks or on a frame at the desired place, and muslin strips are hung over the frame dipping into the pan, which is filled with water. This is the iceless refrigerator designed by Hunt (*6*). If the apparatus is shaded from the sun and the pan kept filled with water, a very efficient damp chamber will be provided for field work. Several methods of "inoculation" were tried, aecidiospores in each case being obtained from wild blackberries growing at Radnor Heights, near Rosslyn, Va.

AECIDIOSPORES SOWED ON SHOOTS

Leaves bearing aecidia were laid over young shoots, so that spores were shed naturally over growing tips, etc., or the spores were dusted over the shoots and into the leaf axils. In this way an excess of moisture which prevents proper germination is avoided. Aecidiospores were also sprayed on the shoots or injected into growing tips, leaf axils, etc.

SPORIDIA ATOMIZED ON SHOOTS

Aecidiospores were germinated on water in watch glasses, or on agar, and the sporidia thus obtained in large numbers were sprayed on the young shoots. It is clear that the use of sporidia instead of aecidiospores will prove to be more satisfactory in infection experiments since the time during which the plants must be kept in the damp chamber is shortened by at least 24 hours. In the writer's experiments, plants are

kept under the infection frame two days when sporidia are used, and three or four days when aecidiospores are sowed.

INOCULATION BY MEANS OF THE HYPODERMIC NEEDLE

A number of attempts were made to infect young shoots by injecting spores into their growing tips with a hypodermic needle. In several cases infection followed in spite of, rather than because of, this method. The needle was loaded with an abundance of sporidia and generous doses were given the young shoots. Water containing sporidia must have trickled from the wounds over the surface of the young shoots, which were exposed by digging away the surrounding soil. The injury to the growing tips manifested itself the following year. The ends which had been punctured usually died and new shoots developed from below.

INOCULATION IN THE OPEN

During rainy weather when the plants remained moist for the greater part of a day or two, inoculations were made with great success by spraying young shoots with sporidia without the use of artificial methods for maintaining the humidity. These experiments were conducted somewhat apart from those which were more nearly under control through the use of muslin infection frames.

CONTROLS

For reasons which will be made clear later, definite control plants were not set aside in this work, although all plants of the same horticultural variety in near-by hills could be considered as control plants, especially where no damp chambers were used. Plants in well-cultivated fields do not serve well as controls since so many of the young shoots are intentionally destroyed in cultivating, and these young plants would be the ones most readily infected by spores from wild blackberries in the vicinity. The destruction of the young shoots that grew up between the hills and rows in the field was avoided in order that use might be made of as many of them as possible as an additional check on the results. Most of the infection experiments with blackberries for 1921 were made in the field plots where exposure to natural infection must be taken into consideration, and it should be remembered that greenhouse conditions in this regard are only slightly more dependable in a region where the orange-rust is so common that vast numbers of spores are very likely to be blown into the ventilators of a greenhouse. One can not be quite certain, therefore, in this vicinity that any particular plant may not have been infected by these wind-borne spores.

It has been shown previously how the rust may be carried from plant to plant through the root runners, so in a group of wild blackberries in which a large number of the plants are rusted it may be that the number of infected plants had increased year by year, as roots containing mycelium gave rise to new shoots. A single primary infection might easily account in this way for conditions where nearly every plant in a stand of wild berries is rusted. Therefore, as an additional safeguard after the writer's plants had been received from nurseries during April and May, 1920, and also in 1921 when the first inoculation work was done, a careful inspection was made from day to day. The work was, therefore, begun with plants whose canes showed no rust the first and second seasons of their growth. For example, a stock plant set out in 1920

develops a horizontal root 1 or 2 feet long, from which arises, in 1921, a shoot in condition for infection. If inoculation is successful, it will be manifested about April, 1922, by the appearance of rusted shoots from the base of the 1921 plant. If examination of sections of the horizontal root connecting the parent plant with the one inoculated fails to show hyphae, it is certain the rust did not come from the parent.

The mere fact that a plant shows no rust on its leaves is not proof that the mycelium is not well established in its crown or in the root runners; but if the runner bearing an infected cane shows no mycelium, the infection must be a new and independent one, regardless of the fact that the parent plant may also be rusted. In this way it will be shown that a root runner may bear two or three infected plants along its course and each plant be separately and independently infected.

Inasmuch as potted plants can not send up shoots from roots at an appreciable distance from the parent plant, they are not as satisfactory for infection experiments as are those grown in "flats" on the bench or as are field plants where shoots are formed on roots 2 or 3 feet from the parent. The Iceberg variety appears especially favorable for the production of root shoots. By digging in the soil, still younger shoots can be exposed when they are in a very susceptible condition.

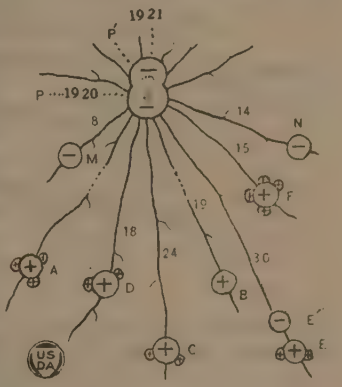

FIG. 4.—Diagram showing arrangement of inoculated plants and roots connecting them with parent Crystal White blackberry, No. 134. Nursery stock, P, P', planted in 1920. Other plants indicated, larger circles, were young shoots in May, 1921, when the experiment was made; small circles attached, new shoots, 1922. Infection frame probably covered area about NEA. Numerals represent distances in inches. Signplus + indicates plant infected May, 1922, when dug; roots bearing plants A and B broken off in digging; sign − indicates uninfected plant. See text and figure 5 for further explanation.

As an illustration of this phase of the work attention is directed to figures 4 and 5 which are diagrams of a part of the root system of plant No. 134, May 6, 1922. The inoculation frame would have covered the six plants, figure 4 (+), which became infected and probably took in either M or N (−) which were not infected. Plant P' was not inoculated although it was a young shoot 5 or 6 inches high when the others were inoculated May 23, 1921. Figure 5 shows how clear-cut the original parent nursery stock remained. There are no shoot buds present on it such as are always found on this vertical underground structure when it harbors mycelium of the orange-rust. The roots N and C arise close together, yet C now bears an infected plant while the one from root N is rust free. The convincing evidence that the parent plant, P, P', is not already parasitized is obtained only by making sections of the root bearing the new plant.

RESULTS

When one studies blackberries in nature, he becomes familiar with those which are infected, and can recognize them after the leaves have fallen. Even though the canes may not be particularly stunted, their appearance and the size of the buds are such as frequently to enable one to pick out the diseased plants. The writer's experimental plants were therefore examined with considerable interest at intervals from June until November following the inoculation experiments. Only one plant was found, September 28, which suggested that the inoculation had been

successful, whereas in reality over 120 separate infections had occurred. The failure to pick out the infected plants lay in the fact that it is not until the second year the rust has lived in the blackberry that the canes become so infected econdarily by invasion of mycelium from the peren-

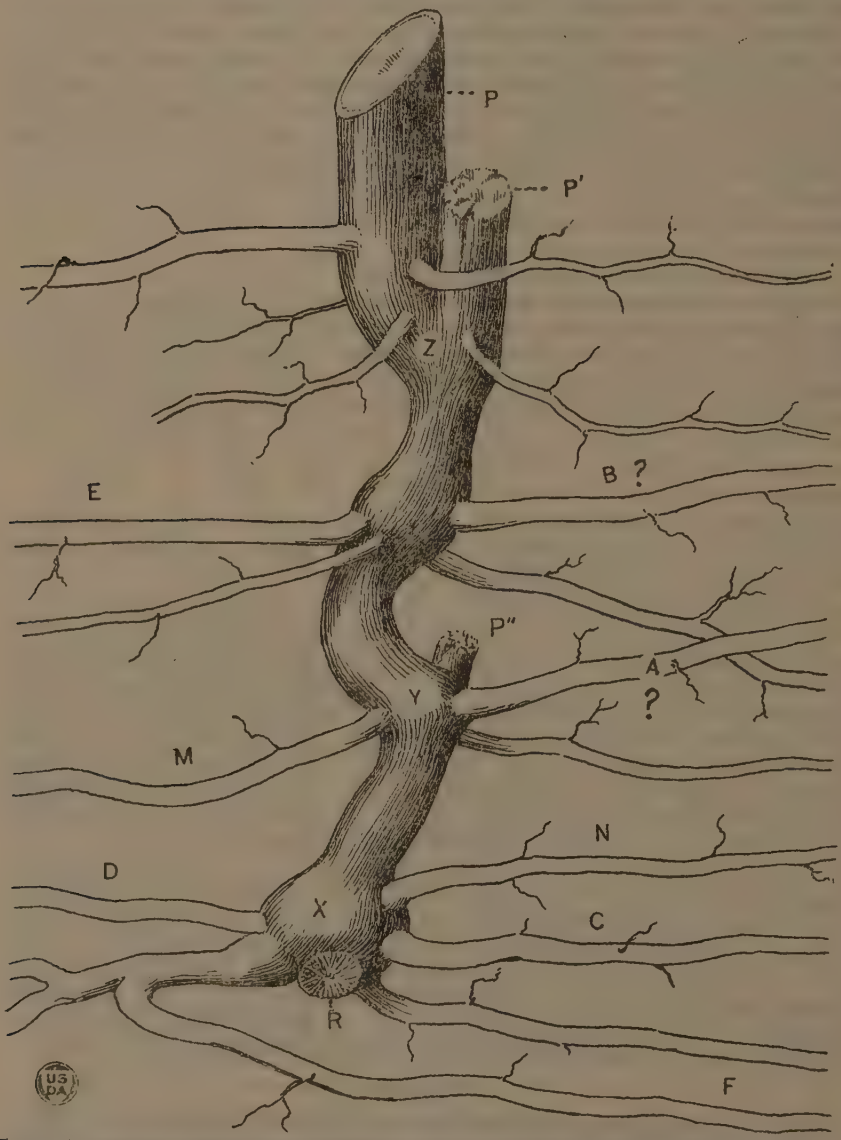

FIG. 5.—Root system of nursery stock of blackberry No. 134 planted May, 1920, dug May, 1922. Lettering has same significance as that in Figure 4. Infected plants A, B, C, D, E, and F arise from roots connected with the original stock at three different levels. The roots shown here are entirely free from mycelium. See figure 4. Original root crown at X, present crown at Z.

nial base that they show the peculiar features mentioned. Canes primarily infected remain for the most part quite normal and blossom as usual, since the upper parts are free from the parasite. The results of the infection experiments are given in Table I.

TABLE I. *Primary infection of the blackberry with sporidia of the short-cycled orange-rust, 1921*

Plant No.	Variety.	Time inoculated (1921).	Infection noted (1922).	Number infected.
92	Eldorado	April 30	April 28	1
93	do	do		0
94	Crystal white	April 23	April 29	2
95 C. W.	do	April 16	May 9	2
97	do	April 30	April 30	4
112	do	May 5	April 28	3
134	do	May 23	May 15	5
336	do	May 2	May 6	2
337	do	April 27	do	3
342	do	do	May 3	5
95	Kittatinny	April 16	April 28	1
98	do	May 2	April 14	9
135	do	May 23	April 28	1
344	do	May 15	April 30	5
353	do	April 20	May 5	2
354	do	April 28	do	2
355	do	April 24	do	2
356	do	April 25	May 16	3
357	do	do	May 5	5
96	Lawton	April 30		0
99	do	May 2		0
101	do	April 23		0
105 B	do	do		0
125 B	do	do		0
136	do	May 23		0
154	do	June 8		0
330	Mercereau	May 2	April 29 May 17	2 5
331	do	April 24	May 20	4
343	do	do	April 30 May 8	2 7
352	do	April 25	May 8	3
369	do	do	May 9	1
370	do	do	do	2
371	do	April 30	May 19	2
372	do	do	May 9	5
373	do	April 26	do	4
377	do	do	May 11	1
333	Iceberg	April 30	May 5	1
334	do	April 20	April 28	5
335	do	April 30	do	1
338	do	do	May 2	2
340	do	May 2	May 1	2
341	do	April 23	May 11	3
345	do	April 27	May 1	1
358	do	April 23	May 3	1
359	do	April 21	May 2	3
360	do	April 26	May 17	3
393	do	May 2	May 25	1
361	Crandall	April 23	May 3	6
415	do	May 2	May 22	1
422	do	do	May 25	1

The results of inoculation work carried on in 1922, repeating many of the experiments reported in the table, furnish further evidence in support of the writer's interpretations and conclusions as regards the various types of infection.

TYPES OF INFECTION

The value of infection work lies not so much in the fact that one acquires the technic of infecting the host, as in the knowledge of the host-parasite relationship to be gained by a study of the plant structures under most favorable conditions. During April and the first week in May, 1922, as an infected plant became noticeable, it was dug up so as to include the root from which it had sprung, and also, in many cases, all other plants connected with the same runner. Pieces of the canes and roots at various points were fixed in Flemming's Medium Fluid and later sectioned and stained. After a careful study of many phases of the question, it was seen that infected plants could be arranged in three or four groups, based upon the manner of the primary attack by the fungus and the reaction of the plant to the attack.

A. This group includes those plants which were, as shoots, infected in or near one or more axial buds below the growing region, so that the shoot grew into a normal cane which blossomed the next spring and would have borne fruit if allowed to live. Rust appeared on the leaves (of the old cane) at a few of the lower nodes as the result of a more or less local infection. New shoots, systemically infected, would arise from the base of the old cane, the one originally inoculated, provided the hyphae had penetrated downward into the underground part of the stem or root. There should be, therefore, in this group three subclasses based on the extent downward to which the hyphae had penetrated: (1) Those infections which were so localized as to be unable to reach the underground parts; the result would be a local infection which would disappear with the natural death of the cane at the end of the season. (2) Those cases where hyphae penetrated through the cambium or phloem down the stem beneath the soil sufficiently far to stimulate the development of new shoots into which the mycelium could penetrate and live over another year. By merely pulling up such canes so that they break off at the root would be all that is necessary to free the plant from the rust. If such a plant is allowed to live through the spring, however, the rust would be able to establish itself permanently in the crown and roots. (3) The third type would include all cases where the root has been reached by the fungus the first season. In such types the connecting horizontal root must be destroyed.

B. The small number falling in the second group includes those plants in which the fungus soon became established in the growing region of the shoot or axial bud, with the result that the whole cane or branch became systemically infected. Such types can easily be recognized the following spring by the appearance of the new shoot which arises at the end of the old cane, or its branch, by proliferation of the terminal bud which has remained alive over winter.

C. The third group includes those types in which the root was directly infected so that the fungus entered the phloem. In such cases the root lay near the surface of the ground. Such a type of infection results in the formation of a witch's broom at once and the rust becomes fairly well established. Since the mycelium in such cases has probably not penetrated either way in the root more than a few inches, the parasite can be destroyed by pulling out the section of the root which bears the infected shoots. Each of these types of infection will now be considered in detail.

PRIMARY INFECTION BY THE SPORIDIA NOT BECOMING SYSTEMIC

Local infection with the sporophytic stage by aecidiospores is, of course, the rule with the Gymnoconia. The question has frequently arisen whether local, as contrasted with systemic gametophytic infection by sporidia, may not sometimes also occur. One frequently sees rusted leaves on certain branches or nodes, while the leaves at all the other nodes of the same cane remain free. The writer made sections of several such naturally infected canes at points above and below the nodes bearing rusted leaves. Having found hyphae in the pith in each case, he came at first to the erroneous conclusion that infection by Kunkelia is always systemic, the rust appearing more tardily on some leaves, or not at all if the mycelium happened not to penetrate the leaf primordium at an early stage. Later an exception was noted which remained a puzzle until the results of inoculation experiments became available. A sand blackberry at Cameron, N. C., was found May 2, 1921, with rusted leaves at only one or two nodes of its single "old" cane, which was otherwise perfectly normal. Sections of the cane at the base and at points above the affected nodes showed no hyphae in the pith, indicating that in nature "local" infections are possible. The proof of such infections has become conclusive as artificially infected plants have been studied. One clear case noticed was that on plant No. 24, a wild blackberry showing no signs of infection February 14, 1921, when it was transplanted. After it was potted it grew vigorously, showing no rust during the year. On March 22, 1921, it was sprayed with sporidia and kept under a damp chamber three days. The plant was taken from the cold frame January 12, 1922. Leaves soon appeared on the old canes and new shoots grew up, but the plant was perfectly normal, no rust having developed. It has been found that when infected plants are kept from year to year, the leaves of new shoots that push out a few days after the plants are brought into the greenhouse are usually covered with pycnia before they unfold. It was, therefore, surprising to find two months later (March 19) that rust was beginning to show on this plant. The tip of the old cane had died during the winter, but from the uppermost living node a new shoot had recently developed. The leaves of this new branch now bore aecidia. Sections at the base of this cane showed no mycelium in the pith, phloem, or cortex. Just below the infected node, however, hyphae were found in the cambium region and along the medullary rays, but none in the pith. A critical study of the distribution of the mycelium in the cane showed that the parasite had originally gained entrance through an axial bud which must have been at least 3 inches above the ground. The end of the cane had died during the winter, but hyphae were making their way slowly down the cane in the region of the cambium, which it was stimulating to renewed growth. This was indicated by a second annual ring of wood which was being formed (Pl. 2, D, y), the cane at this point being larger than at the base. The plant was then set out in soil on the bench in the greenhouse, where it has since grown vigorously without showing any signs of being infected. It is clear that the cutting out of the one cane which showed rusted leaves at a single node freed the plant entirely from the parasite. Had the infected cane been allowed to remain, it is doubtful whether the hyphae could have reached the perennial parts below the ground before the cane would have naturally died. Several other cases,

some of which will now be described, of similar types of local infection were met in connection with inoculations in the field which leave no doubt that "local" infections do take place when they occur at some point on a shoot several inches above the ground.

Two shoots, No. 353 A and B of the Kittatinny variety, about 4 inches high, were sprayed with sporidia May 5, 1921. These grew into large canes 5 feet tall and blossomed profusely in 1922, but no shoots, such as usually characterize infected plants, grew out from the lower parts of the canes during the spring. The fact that both plants had been slightly infected might easily have been overlooked. Each had been infected at a single node, and the fungus had failed to make any material advance upward or downward in the cane. The small branch of 353 A, was only partly infected; several blossoms appeared at the end. The parasite had attacked plant 353 B (Pl. 3) somewhat more vigorously because all of the leaves of two small branches from the infected node showed the rust. Sections of the canes above and below the infected node would show no hyphae. These attacks were very limited in extent and could not have succeeded in permanently infecting the plant. Plate 3, A, shows local infections in a Crystal White blackberry.

Some very striking cases of localized primary infections of the wild dewberry *Rubus enslenii* were noticed at Salem, N. C. Several plants showed rust only on leaves at one or two nodes; the rest of the vine in each case appeared to be perfectly normal. Longitudinal and transverse sections of the vines at about 2 inches above and below the infected nodes were made, but no trace of mycelium was found at these points. There can be no doubt that they were primary infections, and of a local nature, reminding one of the way the Calyptospora attacks the blueberry. The shoots from the infected nodes of these dewberries were very severely attacked. Rust pustules broke out along the young shoots, on the pedicels, and on the calyces as well as on the leaves. The conditions at the time they were infected must have been particularly suitable for the type which the writer calls localized gametophytic infections which fail to become perennial. The same spot was visited in May, 1923, and it was found that the rust had become established in only one of the plants. The shoots must have been rather well developed when the sporidia were being shed.

In 1922 sporidia were sowed on a number of Taylor blackberries whose new canes were from 3 inches to 2½ feet high. This variety proved to be very susceptible. The results indicate still more clearly that canes can be infected with the orange-rust even after they have reached a height of a foot or more. Eight of the canes that showed rust in the spring of 1923 were infected at nodes now 18 inches to 2½ feet above the soil (Pl. 5, B). In some canes the absence of hyphae in the internodes proved that separate infections had occurred at adjacent nodes. It will be shown later that had these plants been grown in flats in a greenhouse, the mycelium would have spread pretty generally through the canes and into the roots because of the conditions which contribute toward etiolation and thus prevent the canes from entering the dormant condition until later. A vine of the dewberry, *Rubus hispidus*, was found infected at only one node. No mycelium was seen in sections of the adjacent internodes. Cutting away the infected node freed the plant entirely. It has grown two years since without showing rust.

LOCALIZED SYSTEMIC INFECTION MAY BECOME PERENNIAL

Slight infections which may become constitutional if the canes are not destroyed are represented by No. 334 F (Pl. 4, A). The shoot from which this plant had developed was originally infected near the soil level after inoculation April 23, 1921. A normal cane developed during the summer. Two or three new shoots, systemically infected, grew from the base of the cane the following spring, and rusted leaves were also noted at the lowest nodes of the old cane May 3, 1922. There were no hyphae in the root, 12 inches nearer the parent plant, which was set out in 1920. This figure illustrates a common habit of growth of such horizontal roots which become larger after having given rise to a new cane. Fully two-thirds of the successfully inoculated plants of the Iceberg and Mercereau varieties had much the same type of infection as the one just described. If one merely pulls the cane from its horizontal root the spring following infection, all of the structures containing the parasite might be destroyed, but if in this case the plant had been allowed to live, the parasite would have invaded new roots and shoots and become thoroughly established. Should the fungus have found conditions more favorable during the summer for the extension of its mycelium downward, the type of infection illustrated by No. 112 C, described below, would have been found. Plate 4, B, illustrates a somewhat vigorous infection of the same type. The tip end of the shoot originally inoculated soon died, but this did not interfere with the activities of the parasite. An axial bud below immediately developed and is now represented by the large cane which is about to blossom, only the basal nodes of this old cane being infected. Three systemically infected shoots arise from the part beneath the soil. If the primarily infected old cane is broken off or cut away during the winter or in the spring before the sap begins to flow, a larger number of new shoots such as are shown in Pl. 5, A, will develop, making a witch's broom type of infection.

PRIMARY INFECTION BECOMES ESTABLISHED IN THE ROOT SYSTEM

Supposing the fungus succeeds in reaching the root crown in its course down the stem, it is known from evidence presented previously that it travels along the root and readily makes its way into the buds, which in due time grow up as plants infected secondarily. To what extent does the fungus work back along the root toward the parent plant? Examinations of sections of roots from a number of independently infected plants show that the fungus does invade the roots in both directions, but that the most progress is usually made outwardly. Occasionally, the reverse is true; the diagram of plant 112–C in figure 6 represents the conditions May 22, 1922, in a plant which was inoculated May 5, 1921, when it was a small shoot. The old canes and branches which grew in 1921, are indicated by shaded lines; the new shoots are shown in outline. The infection has ceased to be local and the fungus has established itself firmly in the root as a systemic parasite. All the leaves on the new shoots and leaves at several nodes of the old canes show rust. Buds are pushing out at the base of the old cane 3 inches below the surface of the soil. Six white shoots (C, C′) are developing; a witch's broom is being formed 2 inches farther back along the root. Sections of the root (C, C′), midway between the main stem and the group of small shoots, show mycelium in the medullary rays in the phloem near the cambium, and some in the cortex. Sections of the root 8 inches to the right of C′ show no mycelium.

As no buds or shoots are found on the main root on the other side, one can be rather sure that the fungus in this case traveled back up the root instead of outward, as might be expected. The fact that the root 8 inches distant did not carry mycelium is positive proof that 112–C was primarily infected by sporidia. There is no apparent reason why the fungus might not continue its growth back toward the parent, and thus a parent plant system might become infected through the rust from its root shoots at some distance away. As one finds, then, two infected plants connected by a root carrying the fungus, it is impossible to determine with certainty after the second year which plant was the one primarily infected.

FIG. 6.—Parasite invades root from stem. Diagram of basal part of plant 112 C. Shaded parts of stem represent 1921 growth; branches unshaded, 1922 growth. Mycelium found in cambium, phloem, and cortex of root between C and C'. No mycelium in root 8 inches to the right of C'. See text.

Where one finds in nature several infected plants attached to the same root runner, sections will usually show hyphae in the connecting root. While the writer has not happened to find in nature cases where no mycelium was present, doubtless independent infections of two or three plants which are derived from the same root runner do occur here also. The rust which occurs on young basal shoots of old plants undoubtedly fails in many cases to invade a horizontal root runner and would thus be unable to reach other plants that had arisen in the same manner. The root system of an infected wild high bush blackberry growing in sandy soil was dug up April 13, 1921. It was found that a rusted plant was attached to a root runner 18 feet distant from the original plant which was now dead and decayed. Its large root, 1½ inches in diameter, however, was still alive. Between these two plants there were two small plants not infected, attached to the same root. Passing out in the other direction from the parent, the root runner was followed 12 feet farther.

Sections of this runner at various points in 30 feet of its course showed no mycelium, and as the only rusted plant connected with this long root was the one farthest from the parent, it is clear that the fungus was in this case unable to go back along the root to any appreciable distance, the mycelium having made but little progress beyond the root crown in either direction.

PRIMARY INFECTION SOMETIMES MANIFESTED ONLY THROUGH ROOT SHOOTS

Attention is called to a type of infection the origin of which is not perfectly clear. The plant, 357 A, appears to have been originally infected so near the base of the shoot that the fungus soon reached the horizontal root, where a slight fusiform enlargement 2 or 3 inches long was developed. The shoot grew into a normal cane which showed no rusted leaves May 5, 1922. Instead, three new shoots, thoroughly infected, had grown up from the root a short distance from the old cane (fig. 7). The buds at the base of this cane show that the fungus was

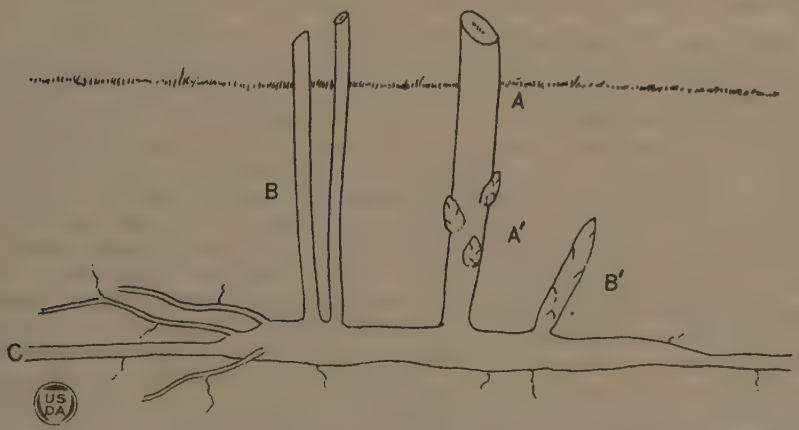

FIG. 7.—Early invasion of root by mycelium from a point originally somewhere near A' results in root infection stimulating growth of shoots B, B' Old cane, A, is normal except for buds, A', which are an indication of the presence of hyphae in that part of the cane.

present in that part which was beneath the soil. The buds would probably have remained dormant, or at least developed only late in the season. The growth of infected shoots on the root at each side of the old cane is against the supposition that the fungus entered the root first through some slight wound, then traveled up the basal part of the cane. The root had given rise in the course of 4 feet to five separate normal shoots, so there is no reason to doubt that the rust now present came as the result of sowing sporidia. A clear-cut case of direct root infection will be described later.

NEW SHOOTS FROM OLD CROWNS SUSCEPTIBLE TO INFECTION

The cases of artificial infection previously described in this paper were of plants which in 1921 were shoots from root runners. In cultivation it is the practice to destroy most of these root shoots which spring up between the rows or between the hills. There is no attempt to destroy the old crown; thus new canes must arise year after year from parts

closely connected with the original crown. Can these new shoots be infected in such a way that the rust becomes established in a plant, the underground parts of which may be several years old? Plant No. 24 (see p. 225), was derived from a wild blackberry crown, potted February 14, 1921. The shoot was infected so near its top that the fungus was unable to reach the underground organs, whereas, if the parasite had entered the shoot when it was just emerging from the soil or at its lowest nodes, hyphae could have penetrated into the underground parts and become firmly established. In some of our experiments shoots from the old underground stem were sprayed with sporidia along with the root shoots. In two instances these shoots became infected. The infection of old crowns, however, by the rust is probably a rare occurrence in nature. When this occurs there will be for several years a number of healthy canes in this hill with only one or two canes showing rust, whereas if the nursary stock or a root shoot is infected the hill derived from either will be worthless from the beginning.

MYCELIUM FROM A PRIMARY INFECTION IN THE GROWING REGION OF THE CANE

Emphasis has been laid upon the point that, in a large percentage of cases observed in our work, the mycelium developing after an inoculation of shoots with sporidia does not travel to any extent up the cane as it grows, but tends to make its way downward into the underground organs, with the result that in the following spring, leaves on new shoots from the base of the cane originally infected and leaves at a few of the basal nodes will develop aecidia, while the cane throughout its upper portion is perfectly normal, bearing blossoms. No mycelium will be found in any of its tissues, except, of course, in the immediate region of the lower rust-bearing leaves. A few notable exceptions to this general rule have been observed. Old canes or branches clearly proved to have been infected as the result of inoculation showed symptoms of having been systemically infected throughout their entire length. Sections of the canes showed mycelium confined to the pith, except at the nodes, just as in canes secondarily infected. Their appearance is quite characteristic, owing to the proliferating terminal and axial buds. Why the fungus behaves in this exceptional way is not clear. No. 135 may be taken as an example of this type of infection. The plant originated as a root shoot from a Kittatinny blackberry 2 feet away. The parent plant has shown no rust for three years. The shoot was about 1 foot high when inoculated with aecidiospores May 23, 1921. On September 28 it was noted that a branch from the base of the plant appeared somewhat abnormal, as though it might be infected. On April 28, the following spring, pycnia appeared on the leaves of the new shoots. On May 8 the infected plant, including the entire root system back to the parent plant, was dug up. The plant now consisted of one large 1921 cane which had been broken off 14 inches above the soil during the preceding summer. Leaves at the basal nodes were rusted and three large new shoots from its base had developed; to this extent the infection appeared to be typical. There was, however, one large 1921 cane (a branch from the lowest node of the cane originally inoculated) which showed peculiarities in that several of its axial buds had been stimulated to grow out into new shoots (Pl. 6, b) and the terminal bud of the 1921 cane had also grown out into a long new shoot, a. This certainly is exceptional behavior for our blackberry, whose

growth is indefinite, the tip ends of the cane being regularly killed at the end of the season. This proliferating tip end was about a foot long. Sections of this 1921 cane 18 inches back of the tip at c showed mycelium only in the pith. The effect of the proliferating shoots along the cane may be seen by the partial second annular ring of wood being formed (Pl. 2, C, y). At the swollen base (Pl. 6, a′) of the 1922 portion, mycelium was also found in the phloem and cortex leading to leaves from this swollen region. Sections of the new growth 2 inches above (a′) showed mycelium only in the pith. Until extended study has been made of the actual point of penetration of the host by the fungus it will be impossible to say definitely when the parasite could have been established in the growing point of this old branch. Mycelium showed only in the pith, except at the nodes where it could be found in the phloem or in the cortex even.

That the original point of infection is not ordinarily the terminal growing region of the shoot is clear from the large percentage of artificially infected canes which show no mycelium in the pith at any point, and none at all in the cane along the greater portion of the upper region. It is possible that where the cane is broken off soon after infection, the axial buds in which the fungus has become established may be stimulated to grow out vigorously, and if the fungus were in the fundamental tissue of the growing region, it would later appear in the pith after tissue differentiation. The writer has observed no case where the main shoot inoculated behaved as though the terminal bud had been attacked, though this no doubt occurs occasionally despite the protection of the growing region, by overlapping leaf primordia. The rapidity with which young canes grow up undoubtedly enables them to outstrip the fungus, which at first seems to grow only slowly, so the mycelium would be left behind in the cortical and phloem regions. Hyphae then make their way slowly up the stem for a short distance and more rapidly downward into the underground stem, and even into the roots. Here the presence of the parasite stimulates the formation of buds, the growing regions of which will be invaded by the hyphae, and as these buds develop into the new shoots the following spring, the fungus, now firmly established, grows upward rapidly and will be found in the pith even at the tip of the new cane.

The writer's experiments show that infection can take place when shoots are several inches high, but in such cases the fungus rarely grows downward with sufficient rapidity in northern latitudes to become firmly established in the perennial underground organs. The younger the shoot inoculated or the nearer the root the infection occurs, the more certain is the fungus to become systemic the following year.

If plants are grown in flats in the greenhouse where the canes are prevented from becoming dormant until late, infection of large shoots at two or three nodes spreads rapidly through the cambium and phloem upward nearly the full length of the stem, and downward well into the root system. Such infections are thoroughly systemic, but no mycelium will be found in the central pith, and there will be no proliferating terminal bud. In the southernmost states where the growing season for blackberries is almost continuous one would expect this type of primary infection to be common in nature.

EFFECT OF INJURY TO THE GROWING POINT

Very frequently when the hypodermic needle is used to inoculate the growing point of a shoot, the injury leads to the death of the end punctured. Sometimes when the soil is being removed from around the root sprouts, the ends will be broken off so that the effect on their future development is practically the same. A new shoot immediately grows out from an axial bud below. The following spring the cane into which the shoot develops will have a short right-angled bend at the point where the scar tissue healed over the end-portion which had died. The plant shown in Plate 4, B, a, also illustrates this feature. On May 5, 1921, the soil was dug up around a plant of the Iceberg variety so that three shoots about 2 inches long were exposed. No. 112 A was inoculated by injecting sporidia into the growing point and spraying them over the surface; aecidiospores were also dusted over the young plant, with the hope that one or the other method might result in infection. No. 112 B and 112 C were merely sprayed with sporidia. The growing point of the first plant which had been injured died, and three new shoots grew out soon after from axial buds below. One of these became the leader and also gave rise to a secondary branch. The leader grew to be 5 feet high and blossomed normally the next year. Leaves at the lower nodes bore the orange-rust May 3, 1922. Four new shoots, systemically infected, now arose from the base of the main cane, but its 1921 branch, as well as the other two canes that were developed in 1921, as noted, showed no rust. The mycelium apparently had not penetrated the horizontal root to any extent, as there were no shoots or buds on the root such as one finds when it is infected. It was found that this root had also given rise in 1921 to the shoot 112 B, mentioned above. Infection resulted from spraying sporidia on the shoot. In May, 1922, it consisted of a single old cane bearing an abundance of blossoms, and the leaves only at the lowest nodes were rusted. New shoots, bearing pycnia were springing up from that part of the cane beneath the soil.

When plants A and B are compared it is seen that the result of killing the growing tip of the inoculated shoot in plant A was simply to stimulate the axial buds to grow into new branches at once, but the type of infection in this case was not altered thereby. Anything that stimulates the development of new and therefore more susceptible shoots or branches from time to time throughout the period of spore dispersal, which varies from two weeks to a month or over, increases the chances of infection proportionately. Very striking effects of accidental layering of *Rubus enslenii* in a neglected cemetery at Winston-Salem, N. C., were observed in April, 1922. A number of graves had been dug the preceding year and the dirt thrown out over these wild dewberry vines. The following spring practically every shoot that was found growing through the covering of dirt from the excavations was rusted. Very few rusted plants were found in this cemetery where the vines had not been disturbed or covered.

ROOTS SUSCEPTIBLE TO INFECTION

One frequently finds orange-rust in hedge rows along the margins of cultivated fields, along embankments, or in pastures, where injury to the canes and roots is very likely to occur. This has suggested the possibility that the blackberry may also be primarily infected through its roots which become exposed through cultivation or otherwise. A few cases of infection were found in the writer's work which were difficult

to explain on any other basis because of the absence of an old cane at the point where infected shoots were then growing. More careful search at such times usually showed the stub-end or scar of a cane which had been cut or borken off accidently or which had died the previous summer. In the absence of such structures through which the parasite might have entered the root, one naturally suspects that the mycelium has traveled along the root runner from some other infected plant. If such a possibility is precluded by an examination of sections, one would have to consider the alternative—direct root infection, which could occur only on exposed roots and before the cork covering had been laid down, or through wounds. For example, in one case it was found that the manner of infection could not be determined at first because of the absence of any remains of, or evidence of, the existence of the orginally infected shoot. Several shoots of the Mercereau (No. 352) blackberry were sprayed with sporidia April 25, 1921. No inoculating chamber was used. On May 8, 1920, aecidia were found on three plants, No. 352 A and B showing the ordinary type of primary infection. Old canes which were rather small and somewhat dwarfed were present and infected shoots were growing from the base of each. There was no old cane nor any trace of a stub or scar showing where one might have been, in connection with plant No. 352 C, the third plant infected. Two new shoots, both infected, and about a dozen buds were growing from a horizontal root where it was somewhat enlarged (Pl. 7, A). Thirty-two inches from these shoots the basal end of an old cane b, was found. The small root runner was followed 14 inches farther where it was attached to a larger root having several branches, c. This root had its origin 18 inches away in a large perfectly normal cane now in blossom. Sections of the horizontal root were made at points as follows: One foot from the parent plant; c_2, 6 inches beyond the stub of the 1921 cane; c_3, 3 inches from the first rusted shoot which was sectioned at c_4. No mycelium was found in the root sections, proving conclusively that the infection had not been carried over from the parent plant. Hyphae were found in the pith only of the infected shoot, c_4, as was expected. It was observed that the second shoot was somewhat woody at its base and had a number of scale leaves about one inch from the point of attachment, indicating that this part of the shoot had been formed the previous summer. This could not have been the shoot originally inoculated and the one which had remained dormant after infection.

It has been noticed that where shoots of the witch's broom type grow up early in the spring they are often woody at the base, which is covered with scale leaves. The buds are forced out the preceding autumn, lie dormant over winter, being protected by a covering of soil, and push up early in the spring. Such conditions are easily found in naturally infected plants. In the case being considered, it is possible that the root had been exposed about the time the inoculation of other shoots had been made and the fungus entered the root probably through a wound. Since shoots devoid of chlorophyll can be most easily infected and since the fungus can thrive in underground parts, there is no evident reason why very young roots may not be directly infected through unprotected epidermis or through wounds extending to the cambium. Evidence in support of such a probability was found in connection with No. 357E, a photograph of which is shown in Plate 7, B. The parent stock, planted in 1920, still bore large healthy canes in 1922. One other old cane

free from rust May 5, 1922, was found attached to the horizontal root 6 inches from the parent crown. Sixteen inches beyond, infected shoots were vigorously developing. Sections of the root at x, y, and z were made but no hyphae were present at these points. No old cane nor any remains of such could be found among the new shoots. There was, however, a very definite scar tissue partially covering an old wound, at w, about an inch long. The root at this point was, when dug, not buried over one-half an inch in the soil, as is clear from the photograph. I do not doubt in the least that the root was originally infected in this wounded tissue.

INFECTION OF BLACKBERRIES IN THE GREENHOUSE

For reasons previously stated, very little work was done in 1921 in attempting to infect blackberries in the greenhouse. As the rust can certainly remain alive in the crown and roots of a plant at least a year without appearing in the canes, one is not certain that his plant, brought in from nature, may not be infected. Such plants should be observed two or three years before being used for experimental purposes, no matter how many controls are used. On the other hand, by careful investigation of the distribution of the mycelium in various structures of an infected plant, one need not remain in doubt, in most cases, as to the time his plant became infected. For example, if mycelium is found in the pith of an old cane whose leaves are rusted the spring following inoculation, it is pretty good evidence that the fungus was present in the plant at the beginning, but if hyphae are found in the phloem, near the outer ends of the medullary rays, or along the cambium or in the cortex, but not in the pith, this will be very good evidence that this cane was infected through inoculation with spores. Only in rare cases does the rust establish itself in the growing point at the time of primary infection. This does sometimes happen, as noted elsewhere, but such cases are very characteristic in their growth the spring following. In the spring of 1922, a number of blackberry shoots growing in "flats" were inoculated with the short-cycled rust. As sections of canes which developed rust the following spring showed no hyphae in the central pith, it is evident that the infections were primary and the result of the inoculation.

PRIMARY AND SECONDARY SYSTEMIC INFECTIONS CONTRASTED

Plants set out in the benches or in large "flats" make root shoots at some distance from the parent plant, but they are not very satisfactory for these experiments. Twelve root shoots of rust-free Kittatinny blackberries were planted in "flats" in the greenhouse April 13, 1922. By May 29 new canes had grown to be from 6 inches to 2 feet high. In order to determine whether canes of such a size could be infected systemically, they were sprayed at this time with aecidiospores of the short-cycled rust from a wild blackberry, and kept in the damp chamber three days. Having been overwintered in the cold frame, they were brought back to the greenhouse March 10. Leaves soon appeared on the old canes which had been inoculated, as noted, the previous year, and new shoots began to grow up from the base of the canes. For at least two weeks all leaves formed were perfectly normal, dark green, hairy on both sides, and not dwarfed. There was nothing abnormal in the appearance of the canes. About March 24 the latest leaves just unfolding at certain nodes became yellowish-green at the margins, which were more finely lobed and wrin-

kled, and devoid of hairs. The petioles tended to elongate abnormally. Some of the axial buds were also beginning to proliferate. The distribution of stomata on the leaves which appeared to be normal and on those showing signs of infection was studied. Practically the same number of stomata was found on the underside of both kinds of leaves. There were only a very few on the dorsal side of the normal leaves, occurring mostly at the tips of the serrations. On the other hand stomata were thickly scattered over the upper epidermis of the abnormal leaves where they showed yellowing. Although no pycnia or aecia are present, the occurrence of large numbers of stomata on the upper side of a blackberry leaf is proof that the orange-rust hyphae have invaded the leaf. The first aecidia appeared April 3. If spermogonia were ever formed on these rusted plants they must have been "vestigial."

The writer had about the same number of systemically infected plants growing under similar conditions at the time, so it was possible to compare blackberries in which the infection had been of long standing, at least two years, with those showing the rust for the first time. The canes of the former were less angular and showed fewer thorns. Pycnia appeared all over the leaves as soon as they began to unfold. Every leaf at each node was dwarfed and yellowish from the start. Stomata were pretty evenly and thickly distributed over the upper surface between the pycnia. Here also it was found that there were about the same number of stomata on the underside of the normal and of the infected leaves per unit area. The total number of stomata per unit area was estimated to be fully 50 per cent greater on the infected leaves. Normal leaves from certain uninfected canes of this plant showed only a few stomata on the upper surface of the tips of the serrations.

A further discussion of the effect of the orange-rusts on the development of stomata is being published in another paper. Unless one were very familiar with the appearance of various types of primary infections, he would have passed over the writer's plants which had been infected the preceding summer because no spermogonia could be found. At the same time the plants that were secondarily infected showed the characteristic symptoms of having the orange-rust.

SPECIALIZED RACES

Since the long-cycled rust is fairly common on black raspberries in localities where the teleutospores have not been found on the blackberries, the possibility of the existence of strains or biologic races should not be overlooked. Whether the blackberry or dewberry can be infected with aecidiospores from the Gymnoconia on black raspberry or *vice versa* has not been definitely questioned by previous investigators. Morphologically the long-cycled rust on black raspberry has not been found different from the rust on blackberry. The writer has made a few preliminary experiments to see how readily the teleutospore stage could be obtained on one species of host by sowing aecidiospores from another variety. These tests are regarded as merely suggestive.

On May 18, 1921, young leaves of new shoots of wild black raspberries at Bell, Md., were inoculated with aecidiospores from the mountain blackberry. On August 20, several teleuto sori were found on one of the lower leaves of one plant. The plants were well isolated in a hedge row 30 rods from any orange-rust. If leaves on old canes had been present when the inoculation was made, no doubt the infections would have been more abundant. Two hybrid blackberry plants, with predominant mountain

blackberry characters, were inoculated with aecidiospores from a wild "yellow" (black) raspberry May 18, 1921. On July 19 many sori were found on the leaves of young canes of one of them. No sori could be found on other plants of the same variety near by. During the spring of 1922 these experiments were repeated more satisfactorily in the greenhouse. Several wild blackberries were planted on a bench. After they had grown out vigorously they were inoculated on several different days with aecidiospores from black raspberries. Spores were rubbed and sprayed on the underside of young and of old leaves, and infected raspberries bearing aecidia were placed under the same infection tent. Thus the blackberry leaves were frequently covered with spores, and conditions were certainly made favorable for infection. At no time later during the summer could telia be found on any of the leaves, old or young, although leaves of the black raspberry plants which had been set in the same infection tents, as noted, were later covered with sori, and they had not been especially inoculated. Four root shoots from these plants were systemically infected with the short-cycled rust in 1922. Such results might be interpreted to indicate that biologic races occur and that the blackberry is immune to the long-cycled rust from the black raspberry, but of course it would first be necessary to prove this particular species or variety of blackberry to be susceptible to infection by the sporophytic stage of the long-cycled rust from other blackberries. Six Kittatinny blackberries were sprayed on May 29, 1922, with aecidiospores from the black raspberry and six others with aecidiospores from a wild blackberry found at Upper Marlboro, Md. On July 19 a few teleuto sori appeared on leaves of each set, and on August 2 other leaves were found well infected with the rust from both sources, suggesting that the Kittatinny variety is susceptible to infection by the long-cycled rust regardless of whether the rust comes from the black raspberry or from this wild blackberry.

It will require much work to settle definitely the question of biologic races of the orange-rusts owing to the difficulties encountered in systemically infecting a susceptible plant with the gametophytic stage. The difficulties can not be overcome nor the questions answered by working with the telial stage alone, because a plant might be susceptible to infection by the telial stage of a certain strain but not be to its orange-rust stage or *vice versa*. The long-cycled rust occurs on blackberries, dewberries, and black raspberries. Can teleutospores be obtained with equal readiness on the dewberry by sowing sporidia from teleutospores obtained from each of the three types of hosts? In spite of the evidence of his preliminary experiments, the writer can not believe that the answer to each question will be in the affirmative.

During the fall of 1920, Mr. George Darrow had some plants of *Rubus canadensis*, the mountain blackberry, sent from Phillips, Me., to Bell, Md., for breeding experiments. They were planted in a plot adjacent to several cultivated varieties. In May 1921, these plants appeared to be seriously affected with the long-cycled orange-rust. During July and August large numbers of teleuto sori were found on the leaves of these mountain blackberries and spores were not rare on the leaves of the Ward, Joy, Mercereau, and the loganberry, showing that in the vicinity of Washington, D. C., the sporophytic stage spreads naturally from the mountain blackberry to several of our well-known varieties of other species. There are at Arlington Farm, Va., some loganberries adjacent to a wild dewberry originally obtained from Phillips, Me., when in an

infected condition. On June 24, 1921, leaves of one of the loganberries whose vines intermingled with those of the infected dewberry, bore large numbers of teleutospores. Another loganberry was inoculated May 23 with aecidiospores from this dewberry. On July 21, teleuto sori were found on several leaves of the loganberry. One of these loganberries had also become naturally infected with the "Kunkelia." It will be interesting to learn whether this variety is susceptible to the gametophytic as well as to the sporophytic stage of the Gymnoconia. It does not follow that a species or horticultural variety is susceptible to the orange-rust stage simply because its leaves can be made to mature teleutospores. Such an example of close heteroecism has not as yet been described, although it may occur. Several plants of the Iceberg variety of blackberry were inoculated with sporidia from aecidiospores from the wild dewberry. Although shoots chosen for this work were in the best condition for infection at the time, no positive results were obtained. This variety is very susceptible to the rust from other blackberries, and probably is not altogether resistant to dewberry rust. More work will be necessary to prove this point.

SUSCEPTIBLE VARIETIES

Plants of different varieties of blackberries and raspberries have been inoculated with aecidiospores of the Gymnoconia without obtaining teleutospores. Some of these failures have been due undoubtedly to faulty technic rather than to the immunity of the host to the sporophytic stage of the long-cycled rust. The Lucretia dewberry certainly approaches complete immunity in North Carolina. The writer has never seen orange-rust on this variety, although no attempts were made in the first experimental work to infect it. Inoculations of the Iceberg, Crystal White, Kittatinny, Mercereau, Blowers, Ancient Briton, and the Crandall with the short-cycled form resulted in such success that the failure to infect a variety of blackberry received from a nursery under the name of "Lawton" suggests that this form, whatever may be its true name, is probably immune to the short-cycled rust. This variety formed so many root shoots in April, 1921, that it was especially chosen for infection experiments without anything being known about its susceptibility. Seven separate experiments in which about 30 shoots were inoculated in various ways were carried out. Since the conditions for infection were fully as favorable as were those which resulted in such marked success with the Kittatinny plants which grew near this "Lawton" variety, it certainly must be very resistant. Several plants of the McDonald were inoculated in the open, but no infection resulted. The attempts to infect the "Lawton" were repeated in 1922 with still greater care but no infections were obtained. The Snyder blackberry also proved to be very resistant. The Taylor, Blowers, and Ancient Briton inoculated at the same time were easily infected.

Fifty separate sowings of sporidia were made on root shoots of cultivated varieties of blackberries in nature in 1921. From 1 to 10 shoots in each case were covered by the infection chamber, so on an average 3 or 4 shoots were inoculated each time. Every attempt to infect the variety, Lawton, as noted above, resulted in failure. Only one plant of the "Eldorado" variety was infected although a number of shoots were sprayed with sporidia. The Eldorado is said to be resistant. At least one shoot of the varieties mentioned was infected in each of the

other 48 trials. The number infected in each case is as follows: Crandall, 8; Iceberg, 23; Crystal White, 26; Kittatinny, 30; and Mercereau, 38. The variety which was shipped to the writer as the Mercereau appears to be the most susceptible. Many infections of this variety were obtained merely by spraying shoots or root sprouts with sporidia without using an artificial humidifier. The results obtained in later experiments, 1922–23, but not included in this summary and not given in the table, furnish further evidence that our commercial varieties of blackberry vary exceedingly in the degree to which they are susceptible to orange-rusts.

The Oregon Evergreen (Black Diamond) blackberry is said to be very resistant to orange-rust, yet the writer found that it could be easily infected by sowing aecidiospores from wild blackberry on its fully expanded leaves, teleutospores developing in about six weeks. This variety, however, may be at the same time very resistant to the gametophytic stage of the long-cycled rust, and to the short-cycled form as well. The southern dewberry, *Rubus enslenii*, which is subject to attack by the short-cycled orange-rust, was readily infected by sowing aecidiospores of the long-cycled Gymnoconia from black raspberry on the leaves. So far as the writer knows, the aecidial stage of this form has not been found on *Rubus enslenii*.

ORANGE-RUST AECIDIA ON CANES AND FLOWERS

If the mycelium, lodged in the perennial underground parts of a blackberry, penetrates a shoot bud and grows up with the cane, this cane, or any part of it bearing hyphae, does not blossom. The localized primary infections described previously are not included in this category, because the mycelium would be unable to reach the growing point and thus grow up with the cane. What at first appeared to be an exception to this rule was noted at Salem, N. C., where several plants of the wild dewberry, *Rubus enslenii*, infected with the short-cycled orange-rust, bore aecidia on the fruit branches, leaf stalks, and even on the calyx of flowers which were being formed in the normal fashion. These were cases of primary infection by sporidia; this was proved by a study of the distribution of the mycelium.

WITCHES' BROOMS ON OLD CANES

The ultimate effect of the orange-rust on most plants if not disturbed by pruning is to cause the canes to become dwarfed or spindling, and to grow out in large numbers from the infected crown. Such brooms should be distinguished from the small ones found at the nodes on certain old canes. It has been thought by some that these distortions are also caused by the rust which is so often found on the leaves at these points. The "double blossom" fungus appears to be able to attack the axial buds of canes infected with the orange-rust so that in the spring both parasites are found together, but it is the "double blossom" fungus that causes the formation of the brooms. These malformations are most common in North Carolina and Virginia, and do not often occur in northern regions on plants infected with the orange-rust, merely because of the absence of the "double blossom" fungus. It is the parasite that lives for the most part on the surface of the organs rather than the one found within the tissues, that stimulates excessive development of buds in old canes.

DISTRIBUTION OF THE ORANGE-RUSTS

The long-cycled Gymnoconia is the only orange-rust known in Europe and Asia. When it was discovered that there were two of these rusts in North America it was said that the short-cycled form was southern in its distribution and the long-cycled strictly northern, the former being the rust so destructive to the blackberries and dewberries grown commercially. It is now known that it is no longer necessary to make pilgrimages to Bartlett, N. H., for the Gymnoconia, because this rust thrives wherever the black raspberry, *Rubus occidentalis*, or its susceptible horticultural varieties may grow. The writer has reported (5) that the rust is common on blackberries near Washington, D. C., and at Old Fort, N. C. He has since found it in abundance on blackberries at Salem, N. C., the type locality of "Kunkelia *nitens*" and at Cornelia, Ga. Germination tests reported in a letter to the author by Dr. Dosdall, mycologist at the Minnesota Agricultural Experimental Station, show that the Gymnoconia is probably very common in that State. No doubt it will be found wherever susceptible blackberries grow. The short-cycled rust, having been recently derived from the other form, is less widely known. Its spread undoubtedly has been accelerated by commercial shipments of diseased nursery stock from one part of this country to another. The temperature ranges through which the Gymnoconia thrives are not different from those suitable for the short-cycled rust, once each is established in its host. One reason why the Gymnoconia follows the black raspberry north and south and why this host is not attacked, at least to any extent, by the short-cycled rust, is that the teleutospores mature at the same time that susceptible tip plants are being developed.

CONTROL OF THE ORANGE-RUSTS

Methods by which the orange-rusts can be eradicated have been suggested in connection with the discussions of the infection experiments. It was pointed out that a blackberry can be freed from the orange-rust very easily if the task is undertaken soon after the primary infection becomes manifest. The mere snapping off of the infected cane at the point of attachment to the root will suffice in many cases. When a number of shoots in the form of a witch's broom are found, it usually indicates that the fungus has invaded the root or its crown; it will then be necessary to destroy this part of the root also. If the primary infection, however, is allowed to spread to the crown and root system the second year, so that new shoots are systemically and secondarily infected, the whole plant must be dug up, care being taken to include the roots for some distance.

It has been shown that it is of the greatest importance to begin a planting with rust-free nursery stock. If the black raspberry to be used has been propagated by rooting the tips of canes one may be reasonably sure of getting some infected plants—that is, if the telial stage of the Gymnoconia is present in the nursery. Whether the tips of canes can be made to root early enough to avoid infection by the first sporidia from teleutospores or late enough so that no buds or shoots that can be infected are formed before the frosts, are problems which will require further investigation. If nurserymen will destroy all infected canes before aecidiospores are shed, there will be no teleutospores in their propagating fields, and it follows that their tip-plants will not be infected when sent to the grower.

So far as controlling the short-cycled rust in the cultivated blackberry is concerned, the writer's experimental work is showing that it is perfectly practicable with a small amount of labor to prevent the spread of the rust. Primary infections by spores occur comparatively rarely in nature; thus, if one observes proper care for a period of two or three weeks in early spring as soon as the first leaves appear, he can readily detect and destroy rusted canes before the mycelium has spread far into the underground perennial structures and before the spores are shed.

The eradication of all rust from a field of blackberries where the disease has been of long standing would be a more difficult undertaking. In New Jersey and in other states one can find fields where from 25 per cent to 75 per cent or more of the plants are infected. Such fields should be planted to some other crop unless the grower is willing to follow up and destroy all roots connected with the rusted plants.

The work at Arlington, Va., reported above, affords a very good illustration of the efficacy of removing infected plants as soon as they show rust for the first time. The writer had about 130 cases of primary infection; wherever the rusted canes were pulled up so as to include all parts of the root runner which showed signs of infection, no rust appeared in 1923. In several cases where it was recorded that undoubtedly pieces of roots bearing mycelium were left in the soil, rusted plants showed in 1923.

Probably one reason why infections by sporidia of the short-cycled rust are comparatively so rare when one considers the vast number of aecidiospores that are matured, is that these spores are rather waxy and therefore, like waxy pollen, are not blown for any great distance by the wind. It would not be good practice, in any event, to allow a rusted plant to remain in the field or to encourage a luxuriant growth of rusted wild blackberries in the vicinity of susceptible cultivated varieties. Two or three days of wet weather at the time new shoots are springing up would certainly result in a further spread of the disease by spores from plants near by. The rust can pass over just as easily from a wild blackberry as from a cultivated variety.

SUMMARY

(1) A study has been made of the distribution of the gametophytic mycelium of the short-cycled orange rust in the blackberry and dewberry, and of the mycelium of the long-cycled rust in the blackberry, dewberry, and black raspberry. In the canes of the blackberry in which either rust has become firmly established as a perennial parasite, hyphae are mostly confined to the central pith and to the fundamental tissue of the growing regions. At the nodes traces of mycelium are sometimes found along the rays in the wood and in the cambium and phloem. Hyphae penetrate the roots very extensively, following the cambium and sieve tubes of the root runner many feet. The cortex is also attcked. Very little mycelium is present in the woody tissue; there is no central pith in the root. New plants arising from the infected root runners will be infected. The spread of the rust from plant to plant in nature occurs frequently through the connecting roots. Mycelium invades the roots and rootlets of infected dewberries very generally but does not follow a root to any great distance. The rust is carried to new plants formed at the rooting nodes by the invasion of these sprouts by hyphae from the vines where the mycelium is distributed in about the same way that it is in the canes of blackberries.

(2) The roots and rootlets of the black raspberry are attacked by the hyphae, mycelium being found not only along the rays but very generally in the wood ring and along the cambium and phloem. As in the dewberry, the mycelium does not follow the roots for very great distances. Hyphae have been found in roots 8 or 10 inches long.

(3) Canes of a thoroughly infected black raspberry do not root at the tips very readily; therefore the long-cycled rust is not so often spread vegetatively to tip plants from an infected parent. The infection of very young tip plants by sporidia from teleutospores largely accounts for the appearance of the rust on new plants. The wild raspberry, *Rubus occidentalis*, and the horticultural varieties, Plum Farmer and Cumberland, were infected by laying black raspberry leaves bearing teleutospores over rooting tips of stolons and maintaining suitable moisture conditions. Infections of the black raspberry also occurred when the teleutospores were taken from blackberry leaves.

(4) Susceptible blackberries can be infected with the short-cycled rust by sowing the sporidia formed on promycelia from germinating aecidiospores on young root shoots; 150 separate primary infections were made in this way. If a blackberry cane has been primarily infected by sowing sporidia, the hyphae of the rust will be found in most cases to be confined to the cambium and phloem tissues. Only rarely do hyphae become established in the tip of an inoculated shoot and grow up with the cane as does the mycelium in a cane from an infected hill. Localized gametophytic primary infections which do not become permanently established sometimes occur, especially if the young cane is several inches high when inoculated. A cane primarily infected usually blossoms normally except at the infected nodes, but canes arising from an infected hill and having mycelium in the growing regions from the beginning do not blossom. Certain nodes may happen to escape invasions by hyphae; in this case blossoms will develop.

(5) Measures for controlling the orange rusts are suggested, emphasis being laid on a thorough inspection of nursery stock for at least one month after planting, and the complete eradication of plants showing rust. Primary infections of blackberry from sporidia are very characteristic and at first do not involve the whole plant and its root system. A limited amount of the root directly connected with the infected cane will usually have been invaded by the hyphae from above, so the destruction of this old cane with a few inches of the root will be sufficient. If such canes are allowed to grow, the parasite soon becomes established in the root crown and it will then be necessary to uproot and destroy the whole plant. As a matter of safety in every case the infected canes and all attached roots should be destroyed.

(6) The infection experiments prove: (a) That the short-cycled rust on wild blackberry can infect such cultivated varieties as the Kittatinny, Iceberg, Mercereau, Crandall, Taylor, Blowers, Ancient Briton, etc.; (b) that the sporophytic stage of the Gymnoconia will go over from the mountain blackberry, *Rubus canadensis*, to such varieties as the Ward, Taylor, Mercereau, and Loganberry, and that teleutospores can be obtained on leaves of certain blackberries and dewberries by sowing aecidiospores from the black raspberry, which can in turn be likewise infected by sowing aecidiospores from the blackberry; (c) that the black raspberry can also be systemically infected with sporidia from teleutospores of the long-cycled rust on blackberry.

We have no reason to suspect that the rust on the wild blackberries is in any way unlike that found on the cultivated blackberries, or that forms of the long-cycled rust on the blackberry and on the black raspberry are at all different biologically, except that certain strains may prove to be more vigorous in their parasitism. As between the systemic stage of the short-cycled and that of the long-cycled rust as they occur on different types of host plants, and as between these and the teleutosporic or sporophytic stage of the Gymnoconia as it is found on blackberry, dewberry and the black raspberry, it must be expected that considerable difference will be found in the readiness with which particular hosts can be infected. Later infection experiments tend to show that the Iceberg blackberry which is very susceptible to attack by the short-cycled rust from the blackberry, is very resistant to the rust from the wild dewberry. This short-cycled dewberry rust may be somewhat different biologically.

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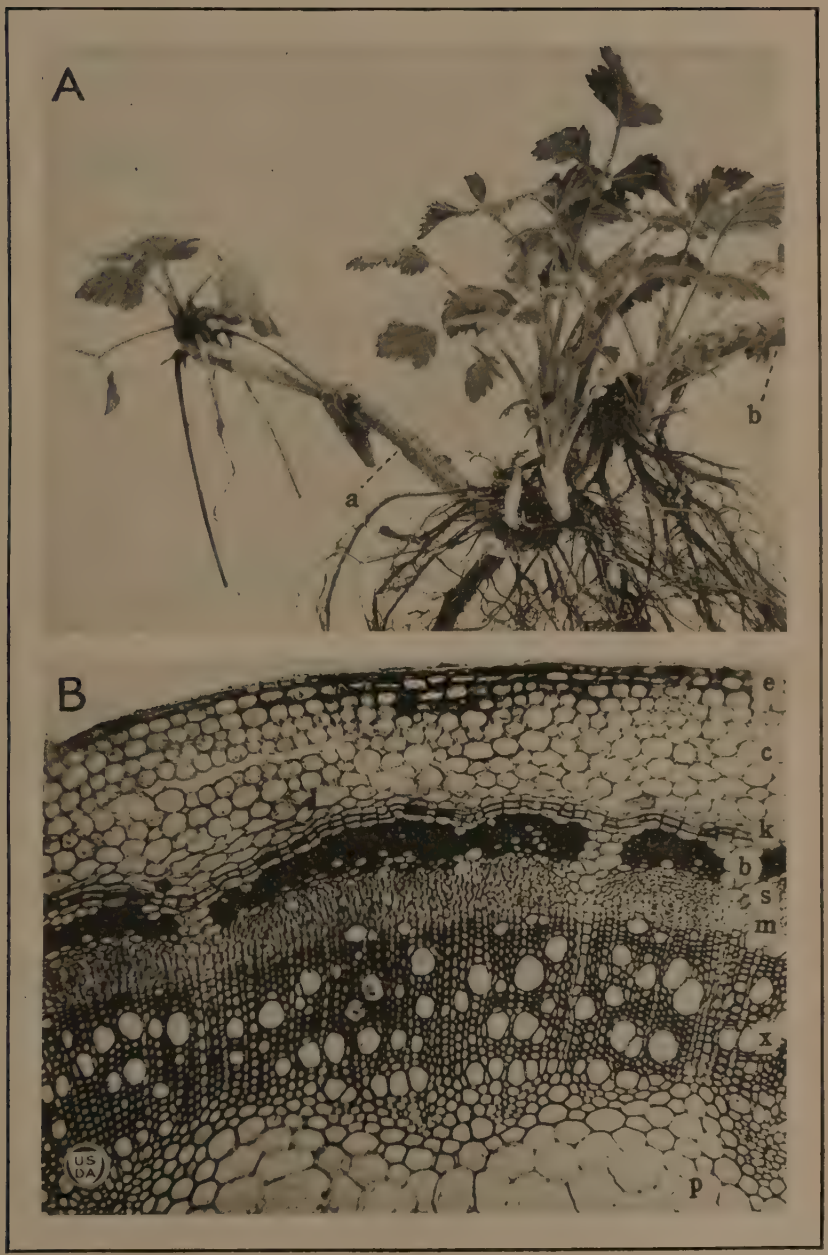
PLATE 1

Rubus occidentalis, black raspberry, infected with the long-cycled orange-rust.

A.—Tip plant which was infected with sporidia of the Gymnoconia in August, 1921; photographed March, 1922.

B.—Section through the cane at b in A: p, pith; x, wood ring or xylem; m, cambium; s, sieve tubes; b, hard bast or stereome (tissues s and b constituting the phloem) k, cork cambium; c, cortex; e, epidermis. Sections of the stolon at a and b in A showed no hyphae; midway between a and b there was an abundance of mycelium in the cambium and phloem, but none in the pith. Sections through the base of each infected shoot revealed hyphae in the pith, phloem, and cortex. See figure 1 for further details.


A
a
b
B
e
c
k
b
s
m
x
p
USDA

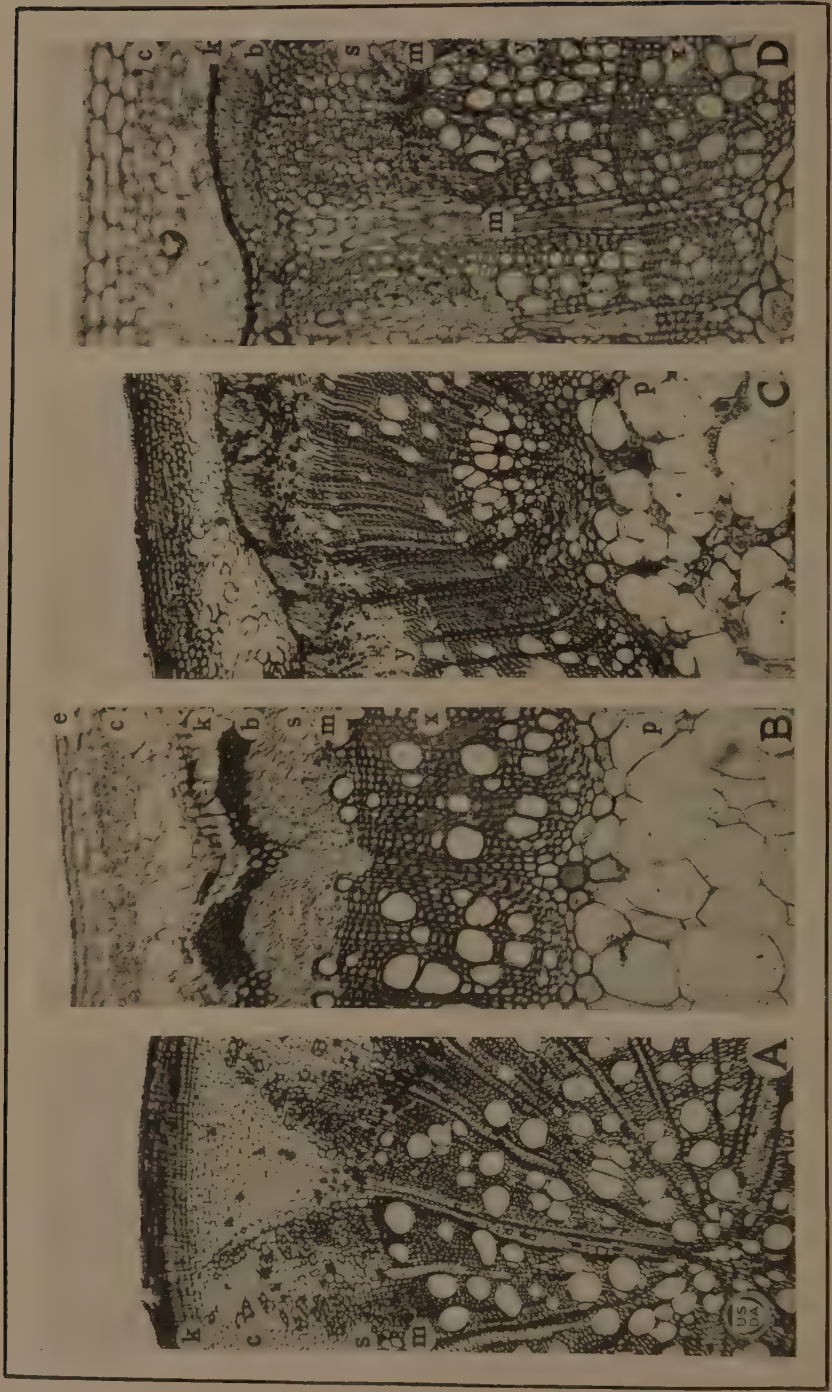

D
C
B
A
c
k
b
s
III
x
p
e
DS
DA

PLATE 2

The small letters have the following significance: p, pith; x, xylem or wood ring; y, second wood ring; m, cambium; s, sieve tubes; b, hard bast or stereome; k, cork cambium or phellogen; c, cortex; e, epidermis.

A.—Cross section of root of blackberry.

B.—Cross section of 2-year-old blackberry cane. Note only one wood ring.

C.—Section of infected cane of blackberry, No. 135, at c in Plate 6, A, just below the proliferating branch, b. Note a partial second wood ring, y. Mycelium was found only in the pith.

D.—Section below a primarily infected branch of blackberry cane, No. 24. The mycelium grew down into the stem along the cambium which was stimulated to form a new ring of abnormal wood and additional phloem. In this section hyphae were ound all through the wood in the second ring, y, and in the rays and phloem.

PLATE 3

A.—Cane and roots of a Crystal White blackberry, No. 342 D, showing localized primary infections on two branches, k and l, of the old cane, uninfected tip ends of cane, and branches cut off. No hyphae were found in sections of the cane at the base, and none in the roots at the cut ends shown here.

B.—Localized primary infection at one node of a cane of a Kittatinny blackberry. Such local infections can not become established systemically because the canes die naturally before the fungus can reach the underground perennial parts of the plant. If such infections occurred on plants grown in the greenhouse or in the southernmost states where the growing season is very long, hyphae would very likely be able to reach the crown of the plant before the cane died.

l
k
A
353
B
B

b
a
A
B
U.S.D.A.

PLATE 4

A.—Common type of primary infection of Iceberg blackberry No. 334 F. The cane above is normal, blossoming profusely, and is free from mycelium. Two or three new shoots are arising from the base of the cane. Hyphae have not yet invaded the base of the cane below the ground, but would have soon done so if the cane had not been destroyed at this time.

B.—Iceberg blackberry in which the growing point had been injured at the time the shoot was inoculated. The present main cane now in blossom, b, arose at once from an axial bud. This cane is free from the rust except at its lowest node. Several infected shoots have developed from the basal part of the cane. The fungus has not yet invaded the root.

PLATE 5

A.—Cultivated blackberry the second year after infection. The old canes are stunted and without blossoms. New shoots are in the form of a typical witch's broom. The longer the rust lives in a plant the more numerous and spindling become the canes.

B.—Taylor blackberry with localized primary infections at the nodes showing etiolated shoots. The upper part of the cane is perfectly normal and would have borne fruit. Such an infection will not become constitutional. The mycelium can not reach the root crown before the cane dies naturally.

A
B
U.S. D.A.

B
A
a
a'
b
c

PLATE 6

A.—Primary systemic infection of Kittatinny blackberry. The axial bud of the shoot inoculated in 1921 developed at once into a branch 2 feet long. The terminus of this branch, instead of being winter-killed, as always occurs in normal canes in our climate, remained alive and its terminal bud (a′) proliferated into a new shoot (a) in 1922. Mycelium was found only in the pith at a in the new shoot and at c in the old cane; at the node between the old and the new growth and where leaves were attached, mycelium was found in the pith and in the medullary ray gaps. A cross section of this cane at c is shown in Plate 2, C; second ring of wood, y, is being developed on the side below a proliferating shoot from an axial bud.

B.—Contrast the type of infection shown here with the localized infection shown in Plate 3. The old cane, originally inoculated when a shoot, had died during the early summer, but the mycelium was soon able to reach the root which lay near the surface of the soil, with the result that several buds were developed along the fusiform enlargement of the root, all of the new shoots being systemically infected. Since the fungus is now established in the runner, the root system must be destroyed to prevent the spread of the parasite. In case of a localized infection at the nodes some distance above the surface of the soil, the rust disappears along with the natural death of the cane at the close of the season.

PLATE 7

A.—Primary root infection of Mercereau blackberry No. 352 C. The parent plant giving rise to the horizontal root now showed a large healthy cane in blossom. A root 18 inches long and one-half inch in diameter branched freely at 12 inches, then gave rise to a slender runner; at b, 14 inches from a, a stub end of a 1921 cane cut off accidentally; 32 inches farther, two new shoots, infected, from a swollen region of the runner. Sections of the root at c, c_2, and c_3 showed no mycelium. Sections at c_4 of the new shoot showed hyphae in the pith only. This is clearly a case of root infection, although no wound scar is visible.

B.—Root attached to No. 357 E, a Kittatinny blackberry showing a case of primary root infection. The parent plant, at the right, was normal, free from rust, and bearing blossoms. Wound callus or scar on the root at w shows where the parasite entered the cortex of the root which lay very near the surface of the ground. No mycelium was found in the horizontal root at x, y, and z. See text for further discussion.

B
z
y
x
w
v
3716
A
c
b
c 2
c 3
c 4
(USDA)